

Chromosomal evolution in Holarctic ground squirrels (*Spermophilus*)

1. Giemsa-band homologies in *Spermophilus columbianus* and *S. undulatus*

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HOWELL (1938) was the first to note morphological similarities between *S. parryii ablusus* and *S. p. lyratus* of Alaska and *S. buxtoni* (= *parryii*) of Siberia and to suggest that the two might prove to be conspecific. Later, HEPTNER (1941) formally suggested that long-tailed ground squirrels of Siberia and northwest North America constituted a single polytypic species with a Holarctic distribution. He included within this Holarctic species Siberian and North American forms of *S. parryii*, as well as *S. undulatus* from western and central Siberia, under the latter name which had priority. That species concept was accepted (RAUSCH 1953; HALL and KELSON 1959) until GROMOV et al. (1963) distinguished between Siberian *S. undulatus* and *S. parryii* on morphological grounds, considering them to be closely related species within the subgenus *Urocitellus*. GROMOV (1965) also included *S. columbianus* from North America in this subgenus. Thus, *Urocitellus* corresponds in part to HOWELL'S (1938) "*Citellus parryii* group".

Analysis of mitotic chromosomes by conventional methods demonstrated $2n=32$ in North American *S. columbianus* and $2n=34$ in North American *S. "undulatus"* (= *parryii*) (NADLER 1966). Later, Siberian *S. undulatus* and *S. parryii* were shown to possess diploid counts of 32 and 34 respectively and karyotypes that were similar to their North American counterparts (VORONTSOV and LYAPUNOVA 1969; LYAPUNOVA 1969). These results supported GROMOV'S (1963, 1965) contention that Siberian *S. undulatus* and *S. parryii* were distinct species. Finally, VORONTSOV and LYAPUNOVA (1969, 1970) suggested that *S. undulatus* and *S. columbianus* probably arose from a common ancestral Holarctic ground squirrel, since the gross morphology of the chromosomes of the species was similar.

Spermophilus parryii, an inhabitant of arctic tundra on both sides of the Bering Strait, would have been able to inhabit the Beringian refugium during the last glacial period (Wurm — Wisconsin). With the waning of that glacial period the land bridge was flooded by rising sea level 12,000 — 13,000 years ago (HOPKINS 1967), and gene exchange between Siberian and Alaskan populations of *S. parryii* must have ceased. In contrast, the intercontinental migration of the ground squirrels ancestral to *S. undulatus* and *S. columbianus* must have occurred much earlier in the Pleistocene. Neither of these species is adapted to arctic tundra and hence could not have utilized the tundra-covered Bering land bridge during the Wurm — Wisconsin or the Riss — Illinoian period preceding it (COLINVAUX 1967). It therefore seems probable that there has been no gene exchange between these populations for at least 100,000 years and perhaps much longer.

Of these three species, *Spermophilus undulatus* occupies the largest geographic range and includes several geographically isolated populations. Two of these are

particularly noteworthy; *S. undulatus jacutensis* occupies a relict steppe area along the left (west) bank of the Lena River in the vicinity of Yakutsk, and *S. u. menzbieri* lives along the Amur River in the vicinity of Blagoveschensk. These two races are widely separated from populations of typical *S. u. undulatus* in the trans-Baikal area and are considerably larger than typical *undulatus*, approaching *S. parryii* in size. This was originally interpreted as representing clinal variation in size from southwest to northeast (HEPTNER 1941; OGNEV 1947) and was in part responsible for the inclusion of *S. undulatus* and *S. parryii* within one species. The discovery of $2n=32$ in *S. u. jacutensis* (LYAPUNOVA 1969) provided strong evidence for placement of the isolate within *S. undulatus*, but the chromosomes of *S. u. menzbieri* remained unstudied.

Conventional methods of chromosome comparison (HOFFMANN et al. 1974) have been hampered by their inability to demonstrate intrachromosomal structure. Recently, however, techniques have been developed that result in differential staining of chromosomes, and homologous chromosome pairs may now be identified by the correspondence of their Q (CASPERSSON et al. 1970), C (ARRIGHI and HSU 1971), or G (SEABRIGHT 1972) banding patterns, according to the method selected. These techniques are adaptable to chromosomal analysis of Holarctic species.

The present study describes the chromosomes of the isolated *S. u. menzbieri* and *S. u. jacutensis* populations from Asia, and compares the G-bands of these populations with those of North American *S. columbianus*.

Materials and Methods

The following 11 specimens were studied: 1. *Spermophilus columbianus columbianus* (Ord); Montana, Madison County, 4.4 km N. of Harrison, 3 females and 1 male; Missoula County, 9 km N. of Missoula, 2 females and 1 male; Canada, Alberta, Highwood Pass, 10 mi S. Kananaskis Lakes, 1 male. 2. *Spermophilus undulatus jacutensis* (Brandt); USSR, west bank of Lena River, 95 km N. of Yakutsk, 1 female and 1 male; 3. *Spermophilus undulatus menzbieri* (Ognev); USSR, Amurskoi Oblast, left bank of Amur River, Zazeiskii Region, 1 female.

Chromosome and Giemsa-band (G-band) preparations were made from bone marrow cell suspensions following intraperitoneal injection of Velban (*S. columbianus*) or coldicine (*S. undulatus*) according to SEABRIGHT (1972). The cells of *S. columbianus* were exposed to 1% sodium citrate for 15 minutes at room temperature instead of the 0.075 M KCl for 5 minutes at 37° C described by SEABRIGHT (1972). Consistent banding patterns were obtained from cell to cell in each species although chromosomes from certain cells exhibited more readily discernable bands than others; no variation in banding patterns between individuals in the same population were found. The diagrammatic representation of the G-bands was derived from 18 metaphase plates in *S. columbianus* and 8 in *S. undulatus*; darkly staining bands are figured as double width whereas lighter bands are drawn in single width.

Results

Spermophilus columbianus from Montana (Fig. 1), *S. undulatus jacutensis* and *S. undulatus menzbieri* (Fig. 2) all possessed a $2n = 32$ and karyotypes indistinguishable from other North American *Spermophilus columbianus* from Idaho (NADLER 1966) and *S. undulatus undulatus*, *S. undulatus stramineus*, and *S. undulatus eversmani* which were analyzed from populations in central Siberia (VORONTSOV and LYAPUNOVA 1969).

The chromosome pairs comprising the karyotypes of these species are readily identifiable on the basis of size and centromere position: pairs 1–4 are large metacentrics or submetacentrics; pairs 5–6 are large submetacentrics; pairs 7–8 are me-

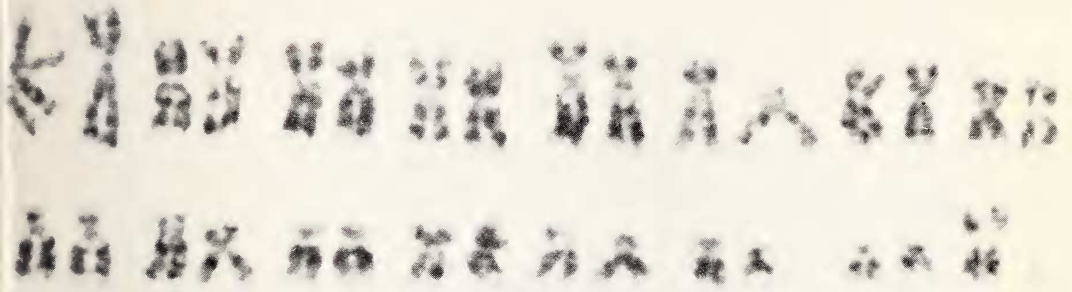


Fig. 1. Karyotype of a male *Spermophilus c. columbianus* from 9 km N. of Missoula, Missoula Co., Montana, stained to show Giemsa bands

dium-sized metacentrics of nearly equal size; pairs 9–12 are medium-sized submetacentric to subtelocentric; pairs 13–14 are small submetacentric autosomes; pair 15 constitutes the smallest pair and is submetacentric; the X chromosome is a medium-sized submetacentric about the same size as pairs 7 and 8, whereas the Y is a minute metacentric. G-band patterns (Fig. 3) greatly facilitated the identification of the chromosome pairs, and in conjunction with chromosome size and morphology assured accurate placement of homologous chromosomes within pairs. The two populations of *S. undulatus* and *S. columbianus* had indistinguishable G-band patterns (Figs. 1, 2).

Discussion

The occurrence of identical diploid numbers and karyotypes, as reflected in common G-band patterns, in *S. columbianus* and *S. undulatus*, is a noteworthy case of karyotype stability during evolution. The ancestral ground squirrel population which give



Fig. 2. Karyotype of a male *Spermophilus undulatus jacutensis* from 95 km N. of Yakutsk, USSR, stained to show Giemsa bands

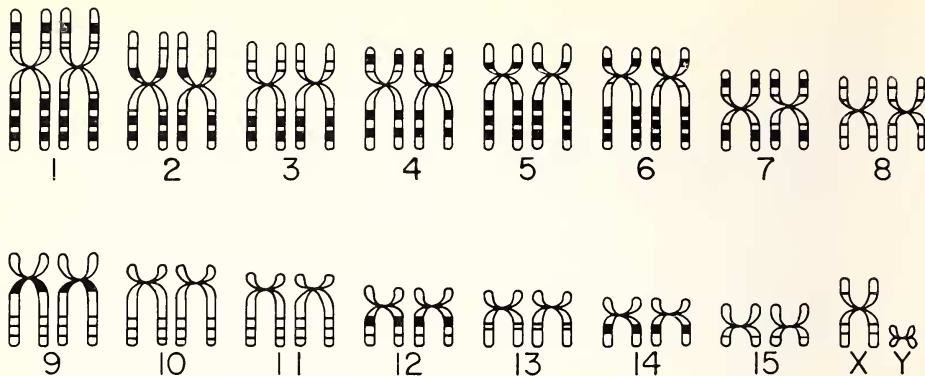


Fig. 3. Idiogram of G-band patterns in chromosomes of *S. columbianus* bases on 18 metaphase cells; patterns in *S. undulatus jacutensis* and *S. u. menzbieri* are indistinguishable

rise to *S. undulatus* and *S. columbianus* once occupied Beringia. This cannot have been during the Wurm-Wisconsin glacial period (70,000–13,000 years B. P.), when the tundra-adapted *S. parryii* lived in Beringia (REPENNING et al. 1964, 1967). Prior to the Wurm-Wisconsin, transberingian dispersal was possible during the Riss-Illinoian glacial period (175,000–100,000 years B. P. HOPKINS 1967), but the climate of Beringia at that time was also harsh (COLINVAUX 1964, 1967). In any event, the populations which gave rise to *columbianus* and *undulatus* must have been separated no more recently than the end of the Riss-Illinoian, 100,000 years ago, and possibly at the end of the Mindel-Kansas glacial period, much earlier. The finding of a fossil long-tailed ground squirrel (*S. undulatus aldanensis*), described by GROMOV (1965), from the east bank of the Lena River, and dated as late middle Pleistocene (equivalent to Mindel-Kansas) confirms the presence of *S. undulatus* in eastern Siberia, adjacent to Beringia, more than 100,000 years ago.

Limited data concerning karyotype stability are available from other taxa of rodents. Several Holarctic species with Beringian distributions at the present day have identical diploid numbers and karyotypes (based on gross morphology). These include the arctic ground squirrel; *Spermophilus parryii*, $2n = 34$ (NADLER 1966; LYAPUNOVA 1969; HOFFMANN et al. 1974); and the tundra vole, *Microtus oeconomus*, $2n = 30$ (RAUSCH and RAUSCH 1968; HSU and BENIRSCHKE 1970). The separation of these species into Siberian and North American isolates by the formation of Bering Strait occurred only about 12,000 years ago (HOPKINS 1967). Populations of the mole rat, *Spalax ebnbergi* ($2n = 60$) from Egypt and Israel respectively, have maintained identical chromosome complements for 10,000–25,000 years (LAY and NADLER 1972).

In the above cases, karyotype stability has been maintained for much shorter periods of time than we are claiming for *S. undulatus* and *S. columbianus*. However, other Holarctic taxa offer indirect evidence for long-time karyotype stability. For example, the common red-backed voles of the north temperate zone are assigned to two species, *Clethrionomys glareolus* in Eurasia and *C. gapperi* in North America both having $2n = 56$ and identical karyotypes (HSU and BENIRSCHKE 1970). These forest-dwelling voles are considered to be closely related (CORBET 1966), but their ecological requirements are such that they probably could not have occupied the Beringian region at any time during the Riss-Illinoian or Wurm-Wisconsin periods. Therefore, these two taxa must have been separated for no less than 100,000 years, and probably more, just as in the case of *S. undulatus* and *S. columbianus*.

This karyotype stability in Holarctic ground squirrels may be contrasted with the significant amounts of divergence that have evolved in other character sets. In external morphology, *S. undulatus* and *S. columbianus* differ in color pattern and tail proportion. Cranial dimensions also differ in the two species and to a greater degree than the cranial differences exhibited by Siberian and Alaskan populations of *S. parryii* (HOFFMANN et al. 1974); ROBINSON 1973). Greater differences in electrophoretic patterns of proteins were also seen between *undulatus* and *columbianus* than between the two *parryii* populations; particularly noteworthy was the occurrence of two unique transferrins (Tf 10, Tf 11) in *S. undulatus* that were not present in *S. columbianus* or any other North American species of *Spermophilus* (NADLER et al. 1973).

It is evident that different character sets in the same species may evolve at different rates, and that genetic differences great enough to separate species may not be accompanied by detectable chromosomal differentiation.

The new chromosomal data presented here support the species affinity of the isolated populations of long-tailed ground squirrels, *S. u. menzbieri* and *S. u. jacutensis*, with other subspecies of *S. undulatus* from the main geographic range studied earlier (LYAPUNOVA 1969; VORONTSOV and LYAPUNOVA 1970). We regard the Yakutsk and Amur isolates as ancient relict forms of *S. undulatus*. However, the habitats of *S. u. jacutensis* and of *S. u. menzbieri* are very different from those occupied by the main populations of *S. undulatus*; the former lives under conditions of the strongly continental climate of central Yakutiya while the latter inhabits the rather rainy and mild climate of the southern Priamur. The ecological and morphological (esp. body size) divergence between these various populations of *S. undulatus* is paralleled by the occurrence of different transferrins. All populations of the smaller animals from the main range (Altai Mountains to Lake Baikal) uniformly possess transferrin 10 (NADLER et al. 1973), whereas *jacutensis* and *menzbieri* have a new, slower transferrin, Tf 11 (NADLER et al. in manuscript).

Although the large body dimensions and identical transferrins might be interpreted as convergence in the two isolated populations, the similarities probably result from a common origin for *S. u. jacutensis* and *S. u. menzbieri*, since the environments they inhabit are so different. Their homozygosity for Tf 11 in comparison to the homozygosity of the Altai-Sayan populations for Tf 10 testify to a prolonged absence of gene exchange between the main geographic range of the species and its northern and eastern isolates. It is possible that a connecting link between the Yakutsk and Amur isolates may have been represented by the large fossil ground squirrel *S. u. aldanensis* Gromov, which formerly lived on the right (east) bank of the Lena along the lower reaches of its tributary, the Aldan, at the end of the middle Pleistocene (see above). We may further assume that the phenetic similarity between *S. u. jacutensis* and *S. u. menzbieri* may date from the time of Samarian (Kansan) glaciation, when, according to VORONTSOV and LYAPUNOVA (1969), *S. undulatus* was widely spread through eastern Siberia, eastward from the Lena. It is likely that geographical barriers (Lake Baikal and glaciers of the northern Baikal plateau) separated *S. undulatus* range into southwestern and northeastern parts during the Riss-Illinoian glacial period; Tf 10 arose in the former and Tf 11 in the latter area.

Genetic data thus indicate the general origin and history of the Amur and Yakutsk populations of *S. undulatus*. It will be of great interest to study transferrin types from populations of *S. undulatus* in Transbaikalia and Vitimsk, localities lying between areas of known Tf 10 and Tf 11.

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Summary

Chromosomes of Eurasian *S. undulatus menzbieri* and *S. u. jacutensis* (both $2n = 32$) are identical to one another both in size and morphology, and in Giemsa band patterns, and are chromosomally indistinguishable from North American *S. columbianus*. Both *S. undulatus* and *S. columbianus* are thought to have evolved from an ancestral species having an amphiberian distribution, and which was subdivided by the Bering Strait at least 100,000 years ago. Although the karyotype has remained stable since the geographic isolation of the Eurasian and North American population, biochemical and morphological divergence has occurred. Although biochemical differences exist between the geographic isolates *S. u. menzbieri* and *S. u. jacutensis*, and populations within the main contiguous range of *S. undulatus*, chromosomes and gross morphology indicate their conspecificity.

Zusammenfassung

Chromosomen-Evolution bei holarktischen Erdhörnchen (Spermophilus). — 1. Homologien der Giemsa-Banden von Spermophilus columbianus und S. undulatus

Die Chromosomengarnituren von zwei isolierten Unterarten *S. undulatus menzbieri* (aus Amurland) und *S. u. jacutensis* (beide $2n = 32$) sind miteinander identisch. Das betrifft nicht nur die Größe der Chromosomen, sondern auch deren Morphologie und Giemsa-Bandenmuster. Diese beiden Unterarten sind ferner in der Chromosomenmorphologie nicht von der nordamerikanischen Art *S. columbianus* zu unterscheiden. Beide Species, *S. undulatus* und *S. columbianus* sind wahrscheinlich aus ancestralen Arten mit amphiberianischer Verbreitung hervorgegangen, welche durch die Beringstraße seit mindestens 100 000 Jahren geteilt ist. Obwohl die Karyotypen seit der geographischen Isolation der eurasischen und nordamerikanischen Population stabil geblieben sind, traten einige Unterschiede biochemischer und morphologischer Merkmale auf. Biochemische Unterschiede bestehen zwar zwischen den geographischen Isolaten *S. u. menzbieri* und *S. u. jacutensis* und bei einzelnen Populationen von *S. undulatus* innerhalb des Hauptverbreitungsgebietes, Chromosomenstruktur und Makromorphologie weisen jedoch auf die Konspezifität dieser Formen hin.

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